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3D architecture of DNA Pol reveals the functional core of multi-subunit replicative polymerases

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision

01 May 2009

Thank you for submitting your manuscript to the EMBO Journal. It has now been seen by three referees whose comments are enclosed. As you can see below, both referee #1 and 3 find the study very interesting, well executed and appropriate for the journal. Referee #2 has no technical concerns with the analysis, but is also not persuaded that the insight provided is sufficient to consider publication in the EMBO Journal. Given the comments and strong support provided by both referee #1 and #3, I would like to invite you to submit a revised manuscript. There are a just few minor concerns that have to be dealt with before proceeding with the publication of your study here.

When you send us your revision, please include a cover letter with an itemised list of all changes made.

Thank you for the opportunity to consider your work for publication. I look forward to reading the revised manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFeree REPORTS

Referee #1:

This manuscript describes the 2.5 Å X-ray crystal structure of the (non-catalytic) C-terminal domain of the catalytic subunit of *Sc* Pol alpha (residues 1263-1468) in complex with residues 246-705 of its non-catalytic B subunit. This structure reveals the nature of the interactions between these two subunits of an essential replicative DNA polymerase. The importance of this information is enhanced by the fact that these interactions are likely to be conserved among the other two major replicative polymerases. Also presented is an EM map of the catalytic subunit of *Sc* Pol alpha (residues 349-1468), again in complex with residues 246-705 of the non-catalytic B subunit. These EM data reveal bilobal molecules in multiple conformations that appear to be connected by an "arm" between the two subunits, both of whose crystal structures can be nicely fit into the EM reconstruction. Collectively, these data provide important structural information on subunit interactions, as well as a larger context for appreciating the function of DNA polymerase alpha, and by extrapolation, the two other essential eukaryotic replicative polymerases. In my opinion, the results are sound, novel, beautifully presented and thoughtfully discussed in the context of existing functional data published by others. In what is a very rare occasion for me, I have no suggestions for change and believe this could be published essentially as submitted.

Referee #2:

In this manuscript the authors used X-ray crystallography and single-particle EM to characterize the structure of the *S. cerevisiae* DNA Pol alpha. A 2.5 Å resolution X-ray structure of the carboxy-terminal domain (CTD) (aa 1263-1468) of the Pol1 catalytic subunit in complex with the C-terminal portion (aa 246-705) of the accessory B subunit provides a detailed description of their interaction. Comparison with the published structure of a delta2-delta3 complex from DNA Pol delta (PDB id 3E0J) suggests that the B subunit functions as a structurally-conserved scaffold for assembly of the multi-subunit DNA polymerases.

The authors also determined a low (~25 Å) resolution 3D EM reconstruction of the Pol1(aa 349-1468)-B(aa 246-705) complex from images of single particles preserved in stain. The EM reconstruction shows a bipartite structure with a smaller lobe that is connected by a flexible linker to a larger domain. The smaller portion of the EM reconstruction matches the shape and size of the X-ray structure of the CTD-B complex and the larger domain matches the published X-ray structure of a *T. gorgonarius* archaeal DNA polymerase/DNA complex (PDB id 2VWJ). Consideration of the archaeal DNA polymerase/DNA complex fit into the Pol alpha EM reconstruction suggested a possible interaction of the Pol alpha B subunit with DNA and the authors present evidence from BIAcore analysis that supports a B-DNA interaction.

The most novel result in the X-ray structure of the Pol alpha CTD-B interface. The EM reconstruction of the Pol alpha Pol1-B complex is at very low resolution. The EM single particle images shown in Supplementary Fig. 2A and the 2D class averages shown in Fig. 5A suggest that the particles selected for EM analysis were rather heterogeneous. However, the EM results largely agree with results (a globular catalytic domain flexibly connected to a more extended assembly of accessory subunits, with DNA extending from the catalytic domain towards the accessory subunits) previously reported in an EM study of *S. cerevisiae* Pol epsilon.

Minor comments:

- Use of the X-ray structure of the archaeal DNA polymerase should be explicitly referenced when the structure is first mentioned (in connection with Fig. 5B and C, where it should be colored differently to distinguish it from the Pol alpha CTD).
- There are several typos and omitted words in the manuscript.

Referee #3:

This an excellent paper that combines crystallography and cryo-EM techniques to provide higher-level structural details of multi-subunit replicative polymerases.

I have only minor suggestions to improve the manuscript.

- p. 6 Rewrite the sentence: "Thus the structure of the B subunit..." which seems odd.
- p. 9 What is the expected error in the estimation of the proportion of the 3 different populations fitting the EM image reconstructions?.

- p. 10 PDB code of pol delta seems wrong (only 3 characters?)
- p. 13: what is the rms deviation to NCS symmetry among the different copies of asu?
- p. 18 Legend of Fig 6: replace "reciprocal" by "respective"?

1st Revision - authors' response

07 May 2009

Referee 1

No points to address.

Referee 2

1. - Use of the X-ray structure of the archaeal DNA polymerase should be explicitly referenced when the structure is first mentioned (in connection with Fig. 5B and C, where it should be colored differently to distinguish it from the Pol alpha CTD).

We have added the reference to the structure of the archaeal polymerase used in the fitting (Firbank et al, 2008) where first mentioned in the text (in the first line of page 9), as suggested by the referee. In Figure 5B, C we have purposely used the same colour for the structures of the archaeal polymerase and the Pol alpha CTD, as they are meant to represent the catalytic and C-terminal domains of Pol alpha. We are concerned that using different colours might confuse the reader and we would prefer to keep the colour scheme as in the original version of the figure.

Referee 3

1. -p. 6 Rewrite the sentence: "Thus the structure of the B subunit..." which seems odd.

The sentence has been modified following the referee's advice.

2. -p. 9 What is the expected error in the estimation of the proportion of the 3 different populations fitting the EM image reconstructions?

In our analysis of Pol α , we have used a maximum likelihood-based classification method that was recently developed to deal with the presence of multiple conformational states (see reference below). Briefly, this classification method searches for the most likely set of parameters to construct a statistical model describing structurally heterogeneous data, through optimization of a log-likelihood function.

In this method, the number of distinct structures is set as an initial parameter. When the classification converges, each particle within the data set is typically assigned to only one 3D class, following the optimization of the log-likelihood function. The number of different classes in the dataset is not normally known at the outset and several classifications using different values must be tested.

The main difference from conventional projection-matching classification methods is that discrete assignments of class membership are replaced by probability-weighted integrations over all possible assignments. Because of this, the proportion of particles assigned to each class is only an approximation and the error in the number of particles assigned to each class cannot be estimated. A summary of the theory behind the method and recent applications can be found in the following publication, cited in the Methods section:

Scheres SH, Gao H, Valle M, Herman GT, Eggermont PP, Frank J, Carazo JM (2007)

Disentangling conformational states of macromolecules in 3D-EM through likelihood optimization. Nat Methods 4: 27-29

We have expanded the legend of Figure 6 in order to clarify the physical meaning of the three 3D solutions obtained by the maximum likelihood method.

3. -p. 10 PDB code of pol delta seems wrong (only 3 characters?)

The PDB code of pol delta p66 is correctly reported as 3E0J.

4. What is the rms deviation to NCS symmetry among the different copies of asu?

Rmsd values between the four chains of the B subunit and pol α CTD in the asymmetric unit vary between 0.08 Å and 0.53 Å. Due to the limited resolution (2.5 Å) of the X-ray diffraction data, NCS restraints were used during the crystallographic refinement. Consequently, the rmsd values between identical protein chains in the asu do not carry physical meaning. The usage of NCS restraints during refinement has now been mentioned in the Methods section.

5. -p. 18 Legend of Fig 6: replace "reciprocal" by "respective"?

We have changed the figure legend according to the referee's indication.

